J. Mol. Biol. (1967) **26**, 361–363

The Absolute Sign of Certain Phase-shift Mutants in Bacteriophage T4

In our original paper on the phase-shift mutants found in the left-hand end of the B cistron of the *r*II locus of phage T4 (Crick, Barnett, Brenner & Watts-Tobin, 1961), the convention for the choice of sign was fixed by chance, the mutant FC0 being arbitrarily allocated sign+. In this short note we discuss evidence which suggests that this sign convention is correct.

Pairs of phase-shift mutants in this region show an unexpected asymmetry. When any pair of phase-shift mutants of opposite sign are combined in one gene, the resulting phenotype mainly depends upon the *order* in which the two mutants occur. Combinations of the type (−+), known as "shifts to the right", usually have a wild, or pseudo-wild phenotype, whereas (+−) combinations (shifts to the left) mostly have the *r* phenotype: that is, the gene does not function.

In a detailed study (Barnett, Brenner, Crick, Shulman & Watts-Tobin, 1967) which presents these facts, we have shown that in most cases in which the double mutant is inactive this is because the phase-shift has produced an "unacceptable" triplet; that is, a triplet which prevents the function of the gene. In the majority of instances we have been able to identify the triplet involved. The details are as follows. For shifts to the left we have located eleven barriers (a barrier is a place which prevents a (+−) or a (−+) combination straddling it from producing an active gene product). The first barrier, b_1, we have not been able to characterize. We suspect it may reflect the peculiar nature of the set of phase-shift mutants to its left at the very beginning of the gene. The last barrier, b_{11}, we have not attempted to investigate. It simply marks the end of the region we have chosen to study. The last barrier but one, b_{10}, we have been unable to identify. It may well be in a region where the amino acid sequence is of some importance. The remaining barriers have been identified as follows (Barnett *et al.*, 1967; Brenner, Barnett, Katz & Crick, 1967):

b_2 UGA
b_3 UAA (ochre)
b_4 UAA (ochre)
b_5 UGA
b_6 UGA
b_7 } leaky
b_8 } UAA (ochre)
b_9 UAG (amber)

All these barriers are single except (b_7 and b_8) which occur as a close pair, one of which is probably leaky and one of which is an ochre. (A barrier is considered single if it can be reverted by a single mutation.)

For shifts to the right, on the other hand, almost any minus mutant combined with any plus mutant to its right gives a gene product which shows activity. The only exceptions are:

(1) A very few plus mutants at the extreme right of the region will not give an active gene unless the minus mutant is fairly close to them.

(2) (−+) combinations which straddle a point about two-thirds of the way along our region are minute on K(λ), showing that the gene is only working very ineffectively. We refer to this latter point as the minute barrier. For technical reasons we have not yet been able to characterize it.

Thus between the extremes of b_1 and b_{11} we have only one doubtful barrier (the minute barrier) for shifts to the right, whereas we have nine barriers for shifts to the left, of which seven are certainly produced by nonsense mutants of one sort or another. Although this could be due to chance, since the numbers are small, it is certainly a little surprising that nonsense mutants are produced so easily by shifts to the left and so rarely, if at all, by shifts to the right.

It has occurred to us that there may be a rather simple explanation for this asymmetry, based on the well-known fact that phage T4 has a DNA which is rich in adenine and thymine, the ratio (A+T)/(G+C) being 1·9 (Wyatt & Cohen, 1953). This makes it likely that, because of the degeneracy of the genetic code (see, for example, Crick, 1966), the third base in any triplet will usually be A or U. Such an expectation is supported by the triplets in the wild-type gene deduced by Streisinger and his colleagues (Terzaghi *et al.*, 1966) from a study of the amino acid changes produced in T4 lysozyme by double phase-shift mutants; all the five triplets allocated by them end in A or U. We may thus write a typical sequence for a piece of T4 messenger RNA as

$$\ldots X_1\ Y_1\ \begin{matrix}A\\U\end{matrix}\ X_2\ Y_2\ \begin{matrix}A\\U\end{matrix}\ldots$$

Thus phase-shifts in one direction will usually give triplets of the form $Y_1\ \begin{matrix}A\\U\end{matrix}\ X_2$, whereas shifts the other way will produce $\begin{matrix}A\\U\end{matrix}\ X_2\ Y_2$. It is obvious that the nonsense triplet UGA can be produced by the shifts of the second type, but not by those of the first.

This explanation accounts very satisfactorily for the asymmetry in the occurrence of UGA, but it does not by itself apply to the ochre triplet UAA or the amber triplet UAG unless we made some *ad hoc* assumption, for example, that perhaps A is rather rare in the third place of the triplets in this region, the third base usually being U or, occasionally, C.

If this explanation of the asymmetry of occurrence of UGA is correct, then we can for the first time deduce whether our sign convention for these phase-shift mutants is correct, or the wrong way round. It is easy to see that the theory implies that our convention is correct. The B cistron is read from left to right, since an amber mutant in the A cistron (which produces polypeptide chain termination) when linked by deletion 1589 to the B cistron, removes the function of the B cistron. Thus, as described above, the actual addition of a single base to the left of any sequence will tend to produce $\begin{matrix}A\\U\end{matrix}\ X_2\ Y_2$ and hence will favour UGA. In practise, our double mutants described as (+−) using our arbitrary convention (that is, having the + on the left) produce UGA. This implies that the addition of a single base would be classed by us as a phase-shift mutant of sign +. It follows that our convention is correct.

Although the argument about ochres and ambers is weak, the argument applied to UGA is fairly strong, even though there are only three examples of it, since it would indeed by surprising if chance effects had produced three instances of UGA when hardly any were expected, and no instances from those shifts which could produce UGA, as would be the case if our convention were the wrong way round.

In conclusion, the asymmetry of the occurrence of UGA produced in our region by double mutants of opposite sign makes it likely that our arbitrary sign convention for these phase-shift mutants is correct.

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Received 28 February 1967

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